

# Reply Exhibit I

**From:** Harwood, Valerie <vharwood@cas.usf.edu>  
**Sent:** Monday, April 21, 2008 1:10 PM  
**To:** Jennifer Weidhaas <jweidhaas@northwind-inc.com>  
**Subject:** RE: QPCR protocol

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Thanks!

Valerie J.(Jody) Harwood, Ph.D.  
Department of Biology, SCA 110  
University of South Florida  
4202 E. Fowler Ave.  
Tampa, FL 33620  
(813) 974-1524 - phone  
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**From:** Jennifer Weidhaas [mailto:jweidhaas@northwind-inc.com]  
**Sent:** Monday, April 21, 2008 2:12 PM  
**To:** Harwood, Valerie  
**Cc:** Tamzen Macbeth  
**Subject:** RE: QPCR protocol

C14 will be shipped later this week.

Roche Applied Sciences, FastStart Universal SYBR Green Master (Rox), Cat. No: 4673484001 (We have also used the non-ROX)  
<https://www.roche-applied-science.com/servlet/StoreFramesetView?langId=-1&krypto=azoH5jhJ2MX7xd3tc73oyEs7A%2BPKEzmv1XJeUzNnNto23xlxth5PYXOI1QAik0yCA8IKLUGWWgyR%0A829tMaxwe%2>

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**From:** Harwood, Valerie [mailto:vharwood@cas.usf.edu]  
**Sent:** Monday, April 21, 2008 12:00 PM  
**To:** Jennifer Weidhaas  
**Subject:** FW: QPCR protocol  
**Importance:** High

Hi Jennifer - Mike has a couple of questions. I think I should be a go-between, that way there can be no question that his lab ever had contact with your lab. I sent him the primer sequences (double-checked them w/the paper and the report). Thanks for your help! I plan to get to that manuscript this afternoon.

His questions:

1. We have 54 samples, including a control. However,

sample C14 is missing? Was this an oversight or

the one that will ship on Monday?

2. We are unsure what SYBR kit

you want us to use. Roche makes many and there is

not one called SYBR Green Master.

In addition, we

are unsure if you want us to use with or without ROX.

Can you please give us the Catalog number of the

SYBR green kit you prefer us to use?

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Hi Jody:

Sorry for the delay, I was out most of the day,

I checked with Dan and yes the second shipment arrived. Sorry, he did not tell me.

We have 54 samples, including a control. However, sample C14 is missing? Was this an oversight or the one that will ship on Monday?

No primers have been sent and we do not have the sequence for these? Can you please let me know what you/they want me to do?

On another matter, we are unsure what SYBR kit you want us to use. Roche makes many and there is not one called SYBR Green Master. In addition, we are unsure if you want us to use with or without ROX.

Can you please give us the Catalog number of the SYBR green kit you prefer us to use?

Thanks for all your help and have a great weekend.

Mike

>Hi Mike - Jennifer said you should have all the  
>samples except one water sample, which they will  
>ship Monday. Have you received ~54 samples?  
>

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>

>-----Original Message-----

>From: Mike Sadowsky [<mailto:sadowsky@umn.edu>]

>Sent: Thursday, April 17, 2008 2:04 PM

>To: Harwood, Valerie

>Subject: RE: QPCR protocol

>

>Hi Jody:

>

>Thanks. Please let me know.

>

>Mike

>

>At 12:01 PM 4/17/2008, you wrote:

>>Hi Mike - finally I have a chance to breathe I

>>just sent a query asking when shipment 2 will

>>come. Thanks for your patience. Jody

>>

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>>-----Original Message-----

>>From: Mike Sadowsky [<mailto:sadowsky@umn.edu>]

>>Sent: Tuesday, April 15, 2008 2:09 PM

>>To: Harwood, Valerie

>>Subject: Re: QPCR protocol

>>

>>Hi Jody:

>>

>>Thanks. I will pass this on to the guys. I got

>>the contract yesterday and am running this through our contracts office.

>>

>>When does shipment 2 arrive?

>>

>>Thanks

>>

>>Mike

>>

>> At 12:04 PM 4/15/2008, you wrote:

>>>Hi Mike - Here is a slight modification to

> the QPCR protocol from Northwind.

>>>

>>>Valerie J.(Jody) Harwood, Ph.D.

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>>>

>>>

>>>-----

>>>From: Jennifer Weidhaas

>>>Sent: Thursday, April 10, 2008 6:44 PM

>>>To: Harwood, Valerie

>>>Cc: Tamzen Macbeth

>>>Subject: RE: DNA

>>>

>>>Jody,

>>>

>>>I just noticed that the qpcr protocol in the SOP

>>>I sent you previously is not our current

>>>method;I have made the revisions to state our

>>>current method in red below. Please forward as

>>>appropriate. We see primer dimer that falls

>>>apart after about 73 deg C and our product is

>>>around 81-82 or so. We have not changed our

>>>method to increase the temperature for the plate

>>>read to remove this primer dimer from the Ct

>>>calculations (rather I removed it by

>>>calculations later) as this is how the first

>>>data set was reported so I didn't want to mess

>>>with the method in the middle. So I am not

>>>recommending that they change the method, but to

>>>be aware of what we have seen. Hope that makes

>>>sense;it has been a long day. Just FYI, all

>>>the fecal samples will be shipped to U of M today.

>>>

>>>Jennifer

>>>

>>>

>>>

>>>1. QPCR PROTOCOL

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>>>1.1 Dilute DNA sample to a final

>>>concentrations of 15 ng / 5  $\mu$ L DNA. Perform the

>>>dilutions in a clean laminar flow hood using aseptic techniques.

>>>

>>>

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>>>

>>>

>>>1.2 Add the following reagents to

>>>each qPCR tube to reach a final reagent

>>>concentration of: 1X SYBR Green Master Mix, 0.5

>>> $\mu$ M 157F and 727 R primer, 5% DMSO, water to

>>>reach 20  $\mu$ L and 5  $\mu$ L of diluted sample DNA.

>>>

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>>>1.3 Operate the Chromo 4 (Bio-Rad)

>>>thermocycler with the following conditions:

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>>>

>>>1.3.1 50  $^{\circ}$ C for 2 minutes.

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>>>

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>>>1.3.2 95  $^{\circ}$ C for 15 minutes.

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>>>

>>>1.3.3 40 cycles of:

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>>>1.3.3.1 95 °C for 30 seconds

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>>>1.3.3.2 60 °C for 30 sec

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>>>1.3.3.3 72 deg C for 30 sec

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>>>1.3.3.4 Plate Read

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>>>1.3.4 50 °C for 5 minutes

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>>>1.3.5 Melting curve:

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>>>1.3.5.1 70 °C to 90 °C by 0.3°C increments

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>>>1.3.5.2 Hold for 5 seconds

>>>

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>>>

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>>>

>>>1.3.5.3 Plate Read

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>>>

>>>

>>>

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>>

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>>Dr. Michael Sadowsky

>>Distinguished McKnight Professor

>>

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